Vascular Risk Factors and Markers of Endothelial Function as Determinants of Inflammatory Markers in Type 1 Diabetes

The EURODIAB Prospective Complications Study

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OBJECTIVE — Inflammatory activity is increased in type 1 diabetes and may predispose to vascular disease. Its origin is not clear. We therefore investigated determinants of inflammation in type 1 diabetes.

RESEARCH DESIGN AND METHODS — We performed a nested case-control study from the EURODIAB Prospective Complications Study of 543 European individuals having type 1 diabetes (278 men), diagnosed at <36 years of age. Case subjects ($n = 348$) were those with one or more complications of diabetes; control subjects ($n = 195$) were all those with no evidence of any complication. We determined levels of C-reactive protein, interleukin-6, and tumor necrosis factor-α, combined them in a "general score of inflammatory markers," and investigated their associations with vascular risk factors and markers of endothelial dysfunction by use of multiple linear regression analysis.

RESULTS — Measures of inflammation were associated with sex, diabetes duration, glycemic control, the advanced glycation end product pentosidine, BMI, HDL cholesterol, triglycerides, and systolic blood pressure (standardized βs with the general score of inflammatory markers 0.15 [$P = 0.002$], 0.15 [$P = 0.006$], 0.18 [$P < 0.0001$], 0.12 [$P = 0.005$], 0.10 [$P = 0.057$], −0.15 [$P = 0.001$], 0.16 [$P < 0.0001$], and 0.09 [$P = 0.042$], respectively). In addition, measures of inflammation were strongly associated with markers of endothelial dysfunction, soluble vascular cell adhesion molecule-1, and soluble E-selectin (standardized βs with the general score of inflammatory markers 0.28 [$P < 0.0001$] and 0.19 [$P < 0.0001$]).

CONCLUSIONS — We have shown that conventional risk factors for vascular disease and endothelial adhesion molecules are important determinants of inflammation in type 1 diabetic individuals, suggesting that strategies to decrease inflammatory activity in type 1 diabetes should focus not only on control of conventional risk factors, but also on improvement of endothelial function.

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Abbreviations: CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; IL-6, interleukin-6; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Atherothrombosis is now widely considered a chronic inflammatory disease. In accordance, plasma markers of inflammation, such as C-reactive protein (CRP) and interleukin-6 (IL-6) levels, are positively associated with risk of vascular disease in nondiabetic individuals (1,2).

Diabetes is an important risk factor for atherothrombosis, an association that is not explained by conventional risk factors. In individuals with type 2 diabetes, inflammatory activity is increased and is strongly associated with risk of atherothrombosis (3,4). Inflammatory activity is also increased in individuals with type 1 diabetes, as shown by increased concentrations of CRP (5,6) and tumor necrosis factor (TNF)-α (7). This elevation of inflammatory markers is thought to be due, in part, to hyperglycemia and the formation of advanced glycation end products (8). However, it is not known what other factors determine the increased inflammatory activity in type 1 diabetes. In particular, the roles of conventional risk factors (2,5,7,9–12), including advanced glycation end products, and of endothelial dysfunction (4,5) have not been clarified. It is also not known whether the presence of microvascular complications influences the associations of conventional risk factors and endothelial dysfunction with inflammatory activity.

In view of these considerations, we investigated determinants of inflammatory activity in the EURODIAB Prospective Complications Study, a representative sample of European individuals with type 1 diabetes (13). Inflammatory activity was estimated from serum concentrations of CRP, IL-6, and TNF-α. We focused on two sets of potential determinants of inflammatory activity, namely, conventional risk factors for atherothrombosis, including estimates of glycemic control and an advanced glycation end product, and markers of endothelial dysfunction (14,15). The latter was esti-

mated from the plasma concentrations of the soluble adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1) and E-selectin.

RESEARCH DESIGN AND METHODS

Subjects

The EURODIAB Prospective Complications Study is a follow-up of the EURODIAB Type 1 Diabetes Complications Study (13). Baseline investigations (1988–1991) were performed on 3,250 men and women with type 1 diabetes from 31 European centers. Sample selection was stratified by sex, age-group, and duration of diabetes, to ensure sufficient representation in all categories. Type 1 diabetes was clinically defined as a diagnosis made before the age of 36 years, with a continuous need for insulin therapy within 1 year of diagnosis. The follow-up (EURODIAB Prospective Complications Study) was performed on average 7–9 years later. Of the 3,250 patients, 1,880 (57.8%) returned for examination (16). At follow-up, a nested case-control study of inflammatory markers was performed (17). This report includes data on 543 individuals in whom serum levels of inflammatory markers (CRP, IL-6, and TNF- α) and endothelial markers (soluble VCAM-1 and soluble E-selectin) were measured.

We assessed micro- and macrovascular complications; did a physical examination; measured height, weight, waist circumference, and resting blood pressure; obtained information on smoking habits; and measured biochemical variables according to a standardized protocol (13). Albumin excretion rates were measured centrally from two 24-h urine collections as previously described (16). Micro- and macroalbuminuria were defined as an albumin excretion rate between 20 and 200 μg/min and >200 μg/min, respectively. Retinopathy was assessed from retinal photographs according to the EURODIAB protocol (18). Cardiovascular disease was defined as a positive medical history of myocardial infarction, angina, coronary artery bypass graft, stroke, and/or ischemic changes on a centrally Minnesota coded electrocardiogram (19).

Laboratory measurements

Follow-up blood samples were sent to central laboratories for analysis. Measurements included total cholesterol (n = 538), HDL cholesterol (n = 537), triglycerides (n = 538), and HbA_{1c} (n = 535) (19). LDL cholesterol (n = 533) was calculated using the Friedewald formula.

CRP (n = 539) was measured with a highly sensitive in-house enzyme-linked immunosorbent assay (ELISA) (5). IL-6 (n = 536), TNF-α (n = 528), soluble VCAM-1 (n = 539), and soluble E-selectin (n = 539) were measured by use of commercially available ELISA kits (R&D Systems, Oxon, U.K.).

Pentosidine levels were determined in unhydrolyzed urine as previously described (20). Urinary excretion of pentosidine was normalized for urine concentration by expressing it as nanomoles of pentosidine per millimoles of urinary creatinine.

Statistical analysis

We employed a nested case-control approach to maximize efficiency. Case subjects were selected to have the greatest complication burden as possible, to provide sufficient numbers for subgroup analyses. Control subjects were selected to be completely free of complications. Thus case subjects were all those with cardiovascular disease or proliferative retinopathy or macroalbuminuria at follow-up, and all those with microalbuminuria and some degree of retinopathy (n = 348). Control subjects were all those who had no evidence of cardiovascular disease, retinopathy, or neuropathy, and were normoalbuminuric at follow-up (n = 195). This selection allowed us to compare individuals with and without complications. Case subjects and control subjects were unmatched, so that the impact of key variables, such as age, could still be assessed, and any adjustments were made at the analysis stage.

All analyses were performed with SPSS 9.0 for Windows 95. CRP, IL-6, TNF-α, triglycerides, and pentosidine had skewed distributions and were natural logarithm (ln)-transformed in all analyses. Linear regression was used to investigate crude (univariate) and adjusted (multivariate) associations of CRP, IL-6, TNF-α, and a general score of inflammatory markers (see below; outcome variables) with vascular risk factors (i.e., age, sex, duration of diabetes, HbA_{1c}, pentosidine, BMI, waist circumference, HDL cholesterol, LDL cholesterol, triglycerides, systolic blood pressure, and pack-years of smoking) and markers of endothelial dysfunction (i.e., soluble VCAM-1 and soluble E-selectin; determinants). In adjusted analyses, we forced all the above vascular risk factors into the model.

We constructed a general score of inflammatory markers that combined information on CRP, IL-6, and TNF-α. For each individual, the value of each inflammation marker was expressed as a Z-score, i.e., [(value in the individual minus the mean value in the study population) divided by the standard deviation], a value that thus ranged from approximately −2.5 to +2.5. The general score of inflammatory markers was then calculated as (Z-score of CRP + Z-score of IL-6 + Z-score of TNF-α)/3.

A P value of <0.05 was considered statistically significant.

RESULTS — Table 1 shows the baseline characteristics of the study population.

For Table 2, we initially performed analyses stratified by the presence or absence of complications. As findings were similar across strata in all analyses, except those involving pentosidine, we combined the groups, except in analyses involving pentosidine.

Table 2 shows the crude and adjusted associations of the inflammatory markers and vascular risk factors. In crude analyses, female sex, age, duration of diabetes, HbA_{1c}, BMI, waist circumference, HDL cholesterol (negatively), LDL cholesterol, triglycerides, systolic blood pressure, and pack-years of smoking were significantly associated with most or all inflammatory markers. Pentosidine was associated with the general score of inflammatory markers similarly in individuals with and without complications, with IL-6 in individuals without complications, and with TNF-α in individuals with complications.

In the adjusted analyses, sex and HbA_{1c} were significantly associated with all inflammatory markers, although the association of HbA_{1c} with CRP was of borderline significance (P = 0.055). Age remained associated with TNF-α levels. Duration of diabetes was associated with TNF-α and the general score of inflammatory markers. BMI and waist circumference remained associated with CRP levels only. HDL cholesterol was negatively associated with IL-6, TNF-α, and the general score of inflammatory markers, whereas LDL cholesterol was indepen-

Table 1—*Characteristics of 543 type 1 diabetic patients*

Variable	Individuals with vascular complications	Individuals without vascular complications	*P*
n	348	195	
Sex (M/W)	185/163	93/92	0.2
Duration of type 1 diabetes (years)	23.6 (19.0–30.3)	13.8 (10.8–18.5)	<0.001
Age (years)	41.8 ± 10.6	36.1 ± 8.1	<0.001
BMI (kg/m^2)			
Men	25.0 ± 3.1	24.5 ± 2.3	0.19
Women	24.7 ± 3.9	23.2 ± 2.8	<0.001
Waist circumference (cm)			
Men	90.0 ± 10.2	88.6 ± 10.0	0.3
Women	81.9 ± 11.9	77.8 ± 9.2	0.002
Smoking status (%)			
Never	35.6	48.7	
Past	31.3	25.6	
Current	33.0	25.6	
Pack-years of smoking			
Past	9.0 (1.0–27.0)	3.3 (0.6–10.3)	0.04
Current	15.0 (7.5–27.5)	9.6 (1.8–16.9)	<0.001
Retinopathy (%)			
No	12.4	—	
Nonproliferative	42.8	—	
Proliferative	44.8	—	
Normoalbuminuria (%)	38.9	—	
Microalbuminuria (%)	24.2	—	
Macroalbuminuria (%)	36.9	—	
Cardiovascular disease (%)	34.8	—	
Systolic and diastolic blood pressure (mmHg)	128 ± 22	115 ± 13	<0.001
	76 ± 12	74 ± 11	0.06
Hypertension (%)	57.5	13.4	<0.001
Serum creatinine (μmol/l)	75 (68–90)	72 (64–79)	<0.001
Total cholesterol (mmol/l)	5.49 ± 1.20	4.97 ± 1.09	<0.001
HDL cholesterol (mmol/l)	1.60 ± 0.43	1.69 ± 0.45	0.02
LDL cholesterol (mmol/l)	3.28 ± 1.10	2.86 ± 0.93	<0.001
Triglycerides (mmol/l)	1.14 (0.85–1.60)	0.84 (0.66–1.08)	<0.001
HbA_{1c} (%)	9.0 ± 1.6	7.7 ± 1.3	<0.001
Urinary pentosidine (nmol/mmol creatinine)	0.47 (0.34–0.68)	0.42 (0.32–0.55)	0.001
Soluble VCAM-1 (ng/ml)	432 ± 142	378 ± 103	<0.001
Soluble E-selectin (ng/ml)	36 ± 17	30 ± 11	<0.001
CRP (mg/l)	1.32 (0.52–2.92)	0.69 (0.35–1.79)	<0.001
IL-6 (pg/ml)	2.14 (1.35–3.96)	1.55 (1.05–2.42)	<0.001
TNF-α (pg/ml)	3.17 (2.35–4.37)	2.23 (1.68–2.85)	<0.001
General score of inflammatory markers (sd)	0.21 ± 0.72	−0.38 ± 0.59	<0.001

Data are *n*, medians (interquartile range), means ± SD, or %. Hypertension was defined as systolic pressure ≥140 mmHg, and/or diastolic pressure ≥90 mmHg, and/or use of antihypertensive drugs.

dently associated only with TNF-α. Triglyceride level was associated with CRP, TNF-α, and the general score of inflammatory markers. Systolic blood pressure remained associated with TNF-α and the general score of inflammatory markers. Pack-years of smoking was not independently associated with any of the inflammatory markers. Pentosidine was independently associated with the general score of inflammatory markers similarly in individuals with and without complications; with IL-6 in individuals without complications; and with TNF-α in individuals with complications.

The percentages of variation (R^2) of CRP, IL-6, TNF-α, and the general score of inflammatory markers explained by the adjusted model were 22.6%, 11.1%, 27.9%, and 26.4%, respectively (Table 2).

Table 3 shows that in crude analyses both soluble VCAM-1 and soluble E-selectin were significantly associated with all inflammatory markers. In adjusted analyses, soluble VCAM-1 was associated with IL-6, TNF-α, and the general score of inflammatory markers, whereas soluble E-selectin remained associated with all inflammatory markers.

Adjustment of the association between vascular risk factors and the inflammatory markers for soluble VCAM-1 and soluble E-selectin did not change the

Table 2—*Crude and adjusted associations of CRP, IL-6, TNF-α, and the general score of inflammatory markers with conventional vascular risk factors*

		CRP		IL-6		TNF-α		General score of inflammatory markers	
	SD	st(b)	*P*	st(b)	*P*	st(b)	*P*	st(b)	*P*
n		515		515		508		508	
Sex (W vs. M)									
Crude		0.21	<0.0001	0.08	0.055	−0.13	0.003	0.08	0.077
Adjusted		0.31	<0.0001	0.13	0.010	−0.12	0.010	0.15	0.002
Age (years)									
Crude		0.20	<0.0001	0.13	0.002	0.10	0.019	0.20	<0.0001
Adjusted	10.1	0.07	0.247	0.05	0.401	−0.17	0.002	−0.03	0.634
Duration of diabetes (years)									
Crude		0.17	<0.0001	0.17	<0.0001	0.25	<0.0001	0.27	<0.0001
Adjusted	9.5	0.01	0.872	0.10	0.101	0.22	<0.0001	0.15	0.006
HbA_{1c} (%)									
Crude		0.15	0.001	0.19	<0.0001	0.28	<0.0001	0.28	<0.0001
Adjusted	1.6	0.08	0.055	0.17	<0.0001	0.16	<0.0001	0.18	<0.0001
Pentosidine (nmol/mmol creatinine)									
Crude		0.08	0.075	—	—	—	—	0.18	<0.0001
Adjusted	1.71*	0.01	0.779	—	—	—	—	0.12	0.005
Individuals with complications†									
Crude		—	—	0.04	0.494	0.25	<0.0001	—	—
Adjusted	1.72*	—	—	0.00	0.957	0.28	<0.0001	—	—
Individuals without complications†									
Crude		—	—	0.26	<0.001	−0.04	0.542	—	—
Adjusted	1.66*	—	—	0.24	0.002	−0.04	0.646	—	—
BMI (kg/m^2)									
Crude		0.33	<0.0001	0.01	0.035	0.14	0.001	0.25	<0.0001
Adjusted	3.2	0.21	<0.0001	−0.01	0.866	0.04	0.458	0.10	0.057
Waist circumference (cm)									
Crude		0.21	<0.0001	0.06	0.149	0.14	0.001	0.19	<0.0001
Adjusted	11.6	0.13	0.025	0.04	0.575	−0.05	0.422	0.06	0.306
HDL cholesterol (mmol/l)									
Crude		−0.06	0.152	−0.15	0.001	−0.21	<0.0001	−0.19	<0.0001
Adjusted	0.43	−0.04	0.368	−0.18	<0.0001	−0.09	0.031	−0.15	0.001
LDL cholesterol (mmol/l)									
Crude		0.13	0.001	0.01	0.885	0.28	<0.0001	0.19	0.001
Adjusted	1.06	0.00	0.953	−0.06	0.237	0.10	0.025	0.02	0.654
Triglycerides (mmol/l)									
Crude		0.23	<0.0001	0.15	0.001	0.37	<0.0001	0.34	<0.0001
Adjusted	1.71*	0.10	0.042	0.04	0.406	0.20	<0.0001	0.16	<0.0001
Systolic blood pressure (mmHg)									
Crude		0.18	<0.0001	0.11	0.014	0.26	<0.0001	0.25	0.0003
Adjusted	20	0.05	0.254	0.03	0.494	0.12	0.008	0.09	0.042
Pack-years of smoking									
Crude		0.11	0.014	0.05	0.231	0.16	<0.0001	0.14	0.001
Adjusted	1.52	0.07	0.098	−0.00	0.930	0.07	0.127	0.06	0.182
Adjusted model (R^2)‡		22.6		11.1		27.9		26.4	

Standardized regression coefficient st(b), i.e., expressed per 1 SD of the independent variable, and *P* obtained by linear regression analyses with the inflammatory markers, CRP, IL-6, TNF-α, and the general score of inflammatory markers as dependent and vascular risk factors as independent variables. *Represents the SD of the geometrical mean. †Stratified when associated different by presence of complications. ‡R^2 represents the percentage of variability of the dependent variable explained by the variables in the model. The adjusted model includes the variables sex, age, duration of diabetes, HbA_{1c}, pentosidine, BMI, waist circumference, HDL cholesterol, LDL cholesterol, triglycerides, systolic blood pressure, and pack-years of smoking (divided into quartiles). The change of the st(b), from + to − after adjustment, in the association of age with TNF-α was caused by adjustment for duration of diabetes.

Table 3—*Crude and adjusted associations of CRP, IL-6, TNF-α, and the general score of inflammatory markers with markers of endothelial dysfunction*

		CRP		IL-6		TNF-α		General score of inflammatory markers	
	SD	st(b)	*P*	st(b)	*P*	st(b)	*P*	st(b)	*P*
n		515		515		508		508	
Soluble VCAM-1 (ng/ml)									
Crude		0.10	0.019	0.21	0.0001	0.49	0.0001	0.40	<0.0001
Adjusted	131	0.06	0.177	0.14	0.002	0.40	<0.0001	0.28	<0.0001
Soluble E-selectin, ng/ml									
Crude		0.12	0.007	0.18	0.0001	0.23	0.0001	0.24	<0.0001
Adjusted	15	0.12	0.005	0.17	0.0001	0.13	0.001	0.19	<0.0001
Adjusted model (R^2)*		24.1		15.2		42.9		36.0	

Standardized regression coefficient st(b), i.e., expressed per 1 SD of the independent variable, and *P* obtained by linear regression analyses with the inflammatory markers; CRP, IL-6, TNF-α, and the general score of inflammatory markers as dependent and soluble VCAM-1 and soluble E-selectin as independent variables. Adjusted model includes the variables sex, age, duration of diabetes, HbA_{1c}, pentosidine, BMI, waist circumference, HDL cholesterol, LDL cholesterol, triglycerides, systolic blood pressure, and pack-years of smoking. *R^2 represents the percentage of variability of the dependent variable explained by the variables in the model including both VCAM-1 and E-selectin.

standardized βs of the vascular risk factors markedly, except for the standardized βs of HbA_{1c}, pentosidine, and systolic blood pressure. HbA_{1c} was associated with CRP, IL-6, TNF-α, and the general score of inflammatory (standardized βs 0.08 [$P = 0.055$], 0.17 [$P < 0.0001$], 0.16 [$P < 0.0001$], and 0.18 [$P < 0.0001$]). Additional adjustment for soluble VCAM-1 and soluble E-selectin weakened these associations (standardized βs 0.04 [$P = 0.30$], 0.11 [$P = 0.01$], 0.08 [$P = 0.02$], and 0.11 [$P = 0.008$]). Pentosidine was associated with the general score of inflammatory markers and, in individuals without complications, with IL-6 (standardized βs 0.12 [$P = 0.005$] and 0.24 [$P = 0.002$]). Additional adjustment weakened these associations (standardized βs 0.04 [$P = 0.34$] and 0.11 [$P = 0.03$]). Systolic blood pressure was associated with TNF-α and the general score of inflammatory markers (standardized βs 0.12 [$P = 0.008$] and 0.09 [$P = 0.04$]). Additional adjustment for markers of endothelial dysfunction weakened these associations (standardized βs 0.04 [$P = 0.30$] and 0.04 [$P = 0.39$]).

The R^2 of the models including further adjustment for soluble VCAM-1 and soluble E-selectin (Table 3) increased versus those without further adjustment (Table 2). Notably, the R^2 for TNF-α increased from 27.9 to 42.9%, and for the general score of inflammatory markers from 26.4 to 36.0%.

Additional analyses

Additional adjustment for serum creatinine levels did not markedly change any of the above analyses, except for the association between the general score of inflammatory markers and systolic blood pressure, where the standardized β decreased from 0.09 ($P = 0.04$) to 0.03 ($P = 0.48$) (other data not shown). Analyses restricted to fasting individuals ($n = 239$) for triglycerides did not materially change the results (data not shown).

CONCLUSIONS — Inflammation is thought to play an important role in atherothrombosis (21). Inflammatory activity is increased in type 1 diabetes (5–7), a disease that confers a high risk of atherothrombosis. It is therefore important to investigate the determinants of inflammatory activity. The present study shows that, in type 1 diabetes, markers of inflammatory activity are associated with sex, diabetes duration, glycemic control, pentosidine, BMI, HDL cholesterol (inversely), triglycerides, and systolic blood pressure. In addition, this is the first study to show that, in type 1 diabetes, inflammatory activity was strongly associated with the adhesion molecules and putative markers of endothelial dysfunction, soluble VCAM-1 and soluble E-selectin. This suggests that endothelial dysfunction plays an important role in the inflammatory activity associated with this disease. This study also shows that conventional risk factors and endothelial dysfunction are important determinants of inflammatory activity regardless of the presence or absence of complications.

Female sex was independently associated with higher levels of CRP and IL-6 but lower levels of TNF-α, in partial agreement with in vitro results (22). A higher prevalence of subclinical urinary infection in women (6) or sex differences in body fat distribution might explain the higher levels of CRP and IL-6 in women (23,24). However, all individuals with urinary infection were excluded from this study. The fact that adjustment for waist circumference did not decrease the effect of sex on CRP and IL-6 argues against the latter interpretation and, in addition, cannot explain the lower TNF-α levels in women. The finding of lower levels of TNF-α is in line with a recently reported in vitro experiment showing that TNF-α secretion from mononuclear cells in response to a lipopolysaccharide stimulus is lower in women than in men (22). The mechanisms behind these sex differences remain to be elucidated.

We show that HbA_{1c} is strongly and consistently associated with all inflammatory markers tested, although a somewhat weaker association with CRP was found. Such associations have been reported previously (7,12,25,26), although those studies did not adjust for other vascular risk factors. The associations between HbA_{1c} and inflammatory activity decreased after adjustment for markers of endothelial dysfunction, which suggests

that poor glycemic control induces inflammatory activity in part through endothelial dysfunction. A close link between poor glycemic control, inflammation, and endothelial dysfunction has also recently been demonstrated in type 2 diabetes (27). HbA_{1c} may reflect the biological activities of hyperglycemia, Amadori products, and advanced glycation end products, all of which can induce inflammation (28,29). In accordance, this study shows that the advanced glycation end product pentosidine was strongly associated with the general score of inflammatory markers, and that this association was independent of HbA_{1c}. The associations between pentosidine and individual inflammatory markers appeared to differ according to complication status (Table 2). There was a strong association with TNF-α in individuals with complications, and with IL-6 individuals without complications, a finding that—in the absence of a plausible biological mechanism, and because of the differences in the association of pentosidine with TNF-α and IL-6—we interpret as due to chance.

Adipocytes can produce IL-6 and TNF-α, and many studies in nondiabetic (9–11,25,26,30,31) and type 2 diabetic (26,32) individuals have shown an association between estimates of body fat and inflammatory activity. Our data show that BMI was associated with all inflammatory markers in crude analyses, but only with CRP in adjusted analyses. The latter is consistent with previous data (5,6,10). It is not clear why associations with IL-6 and TNF-α were weakened after adjustment, but the consistent association with CRP, a strong risk factor for atherothrombosis (33), suggests that even minor increases in body fat may be relevant in young type 1 diabetic individuals.

Levels of triglycerides and HDL cholesterol (inversely) were strongly associated with inflammatory activity, which is consistent with data in nondiabetic individuals (1,11,12). LDL cholesterol was associated with inflammatory activity in crude analyses, but the association disappeared in adjusted analyses. Adjustment for HDL cholesterol and triglycerides may explain this finding. Changes in HDL cholesterol and triglyceride levels often cluster in diabetes. Such unfavorable changes in lipid profile are thought to facilitate the formation of foam cells in the arterial wall, and may thereby increase the inflammatory state in type 1 diabetic individuals. The exact action of HDL cholesterol in preventing cardiovascular events remains to be elucidated (34), but our data lend some support to the concept that the beneficial effect of high HDL cholesterol levels is associated with its anti-inflammatory actions, at least in type 1 diabetes. In addition, we clearly cannot exclude the reverse interpretation, that increased inflammatory activity causes changes in lipid profile (35), as prospective studies are needed to do this. Nevertheless, our data show that in type 1 diabetes, as in type 2 diabetes (25), inflammatory activity and dyslipidemia are clustered phenomena that may both contribute to the risk of vascular disease (36).

Systolic blood pressure was associated with all inflammatory markers in crude analyses and remained associated with TNF-α and the general score of inflammatory markers in adjusted analyses. The prevalence of hypertension in our population was generally high, i.e., 41.7%, owing to the nested case-control approach of this study. High systolic pressure in the vascular tree may damage the endothelial cells and vascular tissue, thereby inducing an inflammatory response (37). Similar associations between inflammation and systolic blood pressure were found in population studies (1,12, 24,30,32).

Somewhat unexpectedly, pack-years of smoking were not associated with any of the inflammatory markers in adjusted analyses, whereas previous studies did find an association between pack-years of smoking and levels of CRP (2,30,38). However, those studies were population-based (2,30) or included elderly individuals only (38), whereas our population is a highly selected group of type 1 diabetic patients.

Markers of endothelial dysfunction were strongly associated with inflammatory activity, which is in accordance with previous findings in type 1 and 2 diabetes (5,39). VCAM-1 and E-selectin enhance the adherence of leukocytes to the endothelium and their transport into the subendothelial intima, where they transform into macrophages and produce cytokines such as IL-6 and TNF-α, which stimulate the production of CRP by the liver. Our data show that concentrations of soluble VCAM-1 and soluble E-selectin, which are thought to reflect their expression on the endothelial cell membrane, contribute substantially to the variation of the general score of inflammatory markers. Therefore, one interpretation of these findings is that endothelial dysfunction causes increased inflammatory activity. Because of the cross-sectional design of this study, we obviously cannot exclude the possibility that inflammation causes increased expression of adhesion molecules and thus endothelial dysfunction (9,21,40). A recent longitudinal study in type 2 diabetic patients has shown that inflammation and endothelial dysfunction are mutually interrelated and progress with time, without one clearly preceding the other (4). This suggests that, in diabetes, inflammation induces endothelial dysfunction (9,21,40) and that endothelial dysfunction may increase inflammatory activity, thus creating a vicious circle (4).

Information about the inflammatory state of an individual can become of clinical relevance since factors that determine inflammation can be modified. Glycemic control, BMI, dyslipidemia, and systolic blood pressure are well-established therapeutic targets in diabetes. Soluble VCAM-1 and soluble E-selectin may be new therapeutic targets. Several substances, such as antioxidants (41), anti–VCAM-1 antibodies (42), and aminoguanidine treatment (43), have been suggested to reduce membrane-bound VCAM-1 expression. Furthermore, statins (44), ACE inhibitors (45), and antioxidants (46) have been reported to reduce soluble E-selectin levels.

A limitation of the present study is its cross-sectional design. However, no longitudinal data of this type in large groups of type 1 diabetes patients exist, and therefore this cross-sectional study may serve as a reasonable starting point to explore these associations in type 1 diabetes. Concentrations of inflammatory and endothelial dysfunction markers were only measured once, which might have diluted the associations we found, which thus may to some extent have been underestimated.

In conclusion, we have shown that conventional risk factors for vascular disease, including glycemic control and an advanced glycation end product, and endothelial adhesion molecules are important determinants of inflammation in type 1 diabetic individuals. These data suggest that strategies to decrease inflammatory activity in type 1 diabetes should focus not only on the treatment of conventional

risk factors, but also on the improvement of endothelial function.

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